

Claims

What is claimed is:

1. A method for determining inhibition of Lp-PLA2 enzyme activity in at least one sample comprising the steps of preparing a solution comprising a substrate for Lp-PLA2 comprising a colorimetric or fluorometric detectable moiety; contacting at least one said sample with the solution of the preparing step; and detecting Lp-PLA2 activity, wherein the sample is from an animal that has been administered with Lp-PLA2 inhibitor.
2. The method of claim 1, further comprising comparing Lp-PLA2 activity from at least one second sample obtained from an animal wherein said second sample is free of said Lp-PLA2 inhibitor.
3. The method of claim 1, wherein inhibition of Lp-PLA2 activity is measured in a plurality of samples obtained from an animal at more than one time point after administration of said Lp-PLA2 inhibitor.
4. The method of claim 1, wherein the substrate is 1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine.
5. The method of claim 4, wherein the 1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine is at a concentration of about 53 μM to about 1125 μM .
6. The method of claim 5, wherein the 1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine is at a concentration of about 440 μM or less.
7. The method of claim 5, wherein the 1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine is at a concentration of about 112 μM .
8. The method of claim 1, wherein the sample is blood plasma.

9. The method of claim 1, wherein the sample is serum.
10. The method of claim 6, wherein the blood plasma is diluted about 3
5 to about 9 fold with the solution of the preparing step.
11. The method of claim 1, wherein the Lp-PLA2 activity is measured by measuring optical density of the sample.
- 10 12. The method of claim 1, wherein the solution comprising a substrate for Lp-PLA2 further comprises a buffer and wherein the buffer is incubated with the substrate prior to contacting the substrate with said sample.
13. The method of claim 12, wherein the buffer does not comprise citric
15 acid monohydrate.
14. The method of claim 1, wherein the substrate concentration is maintained at approximately the K_m of said substrate.
- 20 15. The method of claim 1, the volume of plasma sample is about 15 μL to about 50 μL in a volume of about 125 μL to about 170 μL of the solution of the preparing step.
- 25 16. The method of claim 1, wherein the pH of the reaction is maintained at at least about 7.5 prior to contacting the sample with the solution of the preparing step.
17. A method for determining Lp-PLA2 enzyme activity in a sample obtained from an animal comprising the steps of:
- 30 c) contacting 110 μL of a solution comprising:
a solution comprising 90 mM 1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine contacted with a solution comprising 200mM

HEPES, 200mM NaCl, 5mM EDTA, 10mM CHAPS, 10mM sodium
1-nonanesulfonate at a pH 7.6 in a ratio of 0.66 μ L to 110 μ L;
with at least one 25 μ L tissue sample from an animal;
with 25 μ L each of a p-nitrophenol standard solution comprising; 4,
5 3, 2, 1, 0.4 or 0.2 nmol/ μ L p-nitrophenol in methanol; and
25 μ L of phosphate buffered saline (PBS) or ddH₂O to make a blank;
and

d) determining Lp-PLA2 activity.

10 18. The method of claim 17, wherein the sample from animal is blood
plasma.

19. The method of claim 17, wherein the sample from animal is serum.

15 20. The method of claim 17, wherein the animal is human.

21. The method claim 17, wherein the animal has been administered an
inhibitor of Lp-PLA2 prior to obtaining the sample.

20 22. The method of claim 21, wherein inhibition of Lp-PLA2 enzyme
activity by said Lp-PLA2 inhibitor administered prior to obtaining said sample is
measured by comparing Lp-PLA2 activity of a sample free of said Lp-PLA2
inhibitor.

25 23. The method of claim 17, further comprising:.

e) generating a standard curve by plotting optical density (OD) values at
405 nm for the p-nitrophenol standard solutions vs. p-nitrophenol
(nmol/well);

f) calculating the slope (OD/nmol) of the standard curve;

30 g) calculating absorbance change between 3 and 1 minute ($\Delta OD_{3min-1min}$)
for both solutions comprising tissue samples and blank; and

h) calculating Lp-PLA2 activity using the following formula:

$$\text{Lp-PLA2 activity (nmol/min/ml)} = (\Delta\text{OD}_{\text{sample}} - \Delta\text{OD}_{\text{blank}}) \div \text{slope} \\ (\text{OD/nmol}) \div 0.025 \text{ ml} \div 2 \text{ minutes.}$$